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Review Article

A Review on the *in vitro* Anticancer Potentials of Acetogenins from *Annona muricata* Linn. a Potential Inducer of Bax-Bak and Caspase-3 Related Pathways

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ABSTRACT

Annona muricata Linn. *(A. muricata)* is a tropical evergreen fruit tree belonging to *Annonaceae* family, also referred as graviola, soursop or corossol. The chemical compounds isolated from this plant have linked to the ethnomedicinal properties and its anticancer properties. This review focus to highlight *in vitro* anticancer potentials of acetogenins isolated from *A. muricata*, which are potent inducer of Bax-Bak and Caspase-3 related pathways. More than 200 chemical substances that have been isolated and characterised from this plant and the most significant are alkaloids, phenols and acetogenins. *A. muricata* has a distinct collection of C35 or C37 long chain fatty acid derivatives that are produced from the polyketide pathway and are unique to this family. The main bioactive ingredients bring together many scientific investigations on *A. muricata*, and acetogenins have been the subject of multiple research and reviews. These acetogenins preferentially destroy cancer cells through the action as a DNA topoisomerase I toxin, prevent cancer cells from entering their G1 phase, activate pathways linked to Bax and caspase-3, and block NADH-ubiquinone oxidoreductase (complex I) in mitochondria, while having no impact on healthy cells. In addition to the *in vitro* study, further *in vivo* tests are required to demonstrate these pathways.

Keywords: *Annona muricata*, Acetogenins, Apoptosis, Cancer cell lines.

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INTRODUCTION

Annona muricata (A. muricata), also referred as graviola, soursop, or corossol, is an evergreen fruit tree that is indigenous to the scorching tropical regions of South and North America. Today, it has spread rapidly throughout the tropical and subtropical regions of the world, including Nigeria, India, and Malaysia.[1] Insomnia, cystitis, parasite infections, inflammatory illnesses, neuralgia, and cancer have all been treated using this plant's bark, leaves, and roots. Fevers are widely treated with the fruit and its juice, as well as diarrhoea and dysentery.[2] The lanceolate, glossy, dark green leaves have historically been used as a nervine, sedative, and antispasmodic to treat migraines, hypertension, cough, asthma, and heart conditions.[3] Crushed seeds are used to treat worms, head lice, and other internal and external parasites. The twigs and leaves have sedative and antispasmodic properties.[2] Alkaloids,[4] essential oils,[5] and acetogenins,[6] was discovered in the leaf of *A. muricata*, according to phytochemical analysis. *A. muricata* has a distinct collection of C35 or C37 long chain fatty acid derivatives that are produced from the polyketide pathway and are unique to this family.[7] These phytochemicals have been found to exhibit anticancer effects on their own. At incredibly low dosages, they are selectively toxic to a variety of cancer cells, including cancer cell lines that are resistant to many drugs. The fruit's creamy, decadent flesh is made up of 80% water, 1% protein, 18% carbohydrates, a healthy dose of vitamins B, B2, and C, potassium, and dietary fibre.[8]

The principal bioactive components acetogenins have been the focus of several studies and reviews on *A. muricata* to bring together the diverse scientific inquiries on this plant.[9-12] With selectivity for Prostate Cancer (PC-3) and pancreatic carcinoma, the acetogenins, muricoreacin and murihexocin, produced from the leaves of *A. muricata*, demonstrated considerable cytotoxicity against six human tumour cell lines (PACA-2).[7] It has been discovered that these acetogenins preferentially destroy cancer cells while having no impact on healthy cells.[13] Human pancreatic tumour cell line (PACA-2), human prostate adenocarcinoma

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(PC-3) and human lung carcinoma (A-549) have all been proven to be cytotoxic by acetogenin 1, whilst human hepatoma carcinoma cell line (Hep G2) has been demonstrated to be cytotoxic by acetogenin 2.[14] From best of our knowledge, current review focused on various annonaceous acetogenins for the induction of cancer cell apoptosis through various cancer cell lines.

PHYTOCONSTITUENTS

More than two hundred bioactive compounds have been identified and reported from *A. muricata*. The most common substances among them are acetogenins, alkaloids, and phenols. Due to their long history of usage, leaves and seeds are the primary plant components investigated. The bulk of the phytoconstituents are found in organic extracts, although recently attention has also been paid to aqueous extracts.

ACETOGENINS

In ethanolic, methanolic, or other organic preparations of different parts of *A. muricata* such as leaves, stems, bark, and seeds,[6,15-17] pulp[18] and fruit peel,[11,19] more than 120 acetogenins have been discovered. The *Annonaceae* family's principal bioactive chemicals are known as acetogenins.[15] Acetogenins have a long aliphatic chain of 35-38 carbons connected to a g-lactone ring, which is permanently substituted by ketolactone (β-unsaturated methyl), one or two Tetrahydrofurans (THF) along the hydrocarbon chain, and a certain number of oxygen groups (hydroxyl, acetoxyls, ketones, epoxy) shown in Figure 1.[20] The majority of the acetogenins found in *A. muricata* have a THF ring, but there are also some acetogenins that have two neighbouring or separate THF rings. Acetogenins are linear proteins with one or more epoxy groups. The major bioactive acetogenins isolated from *A. muricata* is given in Table 1.

According to many researches, its bioactivity of acetogenins is influenced by its structure.[2] Annonacin was the typically used acetogenin found in *A. muricata* leaves and fruits[21,22] although it was also found in seeds,[23] peels[19] and roots. Acetogenins in leaves extract was measured using 1H NMR and the estimated quantity varied from 3.38 to 15.05 mg/g, whereas 0.299 mg/g was found using HPLC-MALDI.[24] According to some studies, acetogenins are more cytotoxic than alkaloids and the synthetic cytotoxic agent rotenone. Acetogenins and alkaloids are consequently intensively investigated in a disputed way due to their potential for therapeutic applications versus their neurotoxic activities.

Apart from their distinctive chemical structures, acetogenins have a wide spectrum of bioactivity, including immunosuppressive, antimalarial, insecticidal, antifeedant, and, perhaps most significantly, anticancer properties shown in Figure 2. It has been demonstrated that certain acetogenins can stop the growth of tumour cells that are multiple drug resistant.[32] It is assumed that the mechanism of action of acetogenins is the suppression of NADH-ubiquinone oxidoreductase (complex I) in mitochondria. Apoptosis occurs when ATP synthesis is suppressed, notably in tumour cells with high metabolic rates.

Apoptosis

The crucial stage of programmed cell death known as apoptosis entails significant phenotypic and biochemical alterations that are necessary elements of the process. The optimal stage for cancer therapy is to induce cell death in malignant tissue. Cell cycle disturbance can ultimately result in apoptotic death since apoptosis and cell cycle suppression are closely related. Another successful technique for limiting tumour growth is to stop cancer cells from progressing through their disrupted cell cycle. Chromatin condensation, membrane blebbing, cell shrinkage, and DNA fragmentation are only a few examples of the morphological and biochemical modifications that define apoptosis. For plant products to be effective as anticancer medicines, apoptosis must be induced. Chemotherapeutic agents frequently encourage cell cycle disruption at phase G0 or G1 or phase G2 or M, which can significantly improve their ability to treat cancer.

The potential of the mitochondrial membrane is a traditional indicator of apoptosis, and it is lost when the mitochondrial membrane potential is lost. The quick decrease in membrane potential suggests irreversible early apoptosis brought on by an increase in mitochondrial membrane porosity and the subsequent discharge of apoptotic factors like cytochrome c. There are two primary apoptotic processes, extrinsic via a death receptor and internal via mitochondrial caspase-triggered pathways shown in

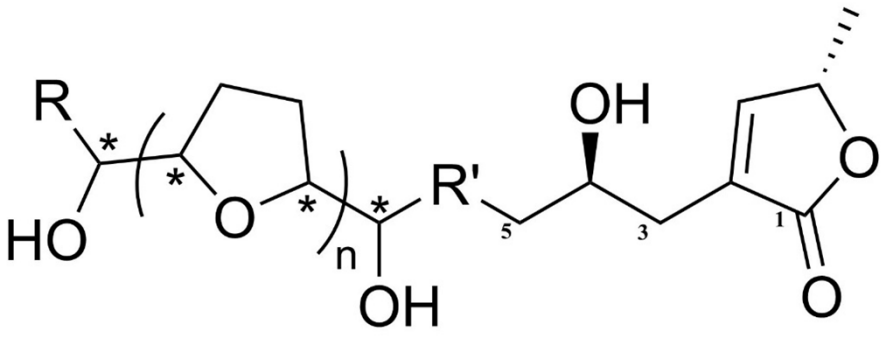

Figure 1: Portraying structure of the annonaceous. R, R'=Hydrocarbon chain with oxygenated moieties and/or double bonds, with *n*=1-3.

Table 1: List of major acetogenins isolated from *A. muricata*.

Acetogenins and Olefinic position	Hydroxyl positions	Relative configuration	Molecular formula	Bioactivity	M^+	References
montecristin, C17=C18; C21=C22 (Linear acetogenins-giganin type)	13,14	threo-cis-cis	$C_{37}H_{66}O_4$	NR	574	25
cohibin-A, C19=C20 (Linear acetogenins-giganin type)	15,16	threo-cis	$C_{35}H_{64}O_4$	NR	548	25
cohibin-B C17=C18 (Linear acetogenins-giganin type)	13,14	threo-cis	$C_{35}H_{64}O_4$	NR	548	25
muridienin-1, C13=C14, 17=C18 (muridienin-1 type)		cis-cis	$C_{35}H_{62}O_2$	NR	514	26
muridienin-2, C15=C16, 19=C20 (muridienin-1 type)		cis-cis	$C_{37}H_{66}O_2$	NR	542	26
epoxymurin-A or epomuricenin-A, C19=C20 (epoxy-acetogenins, epoxymurin-A type)	15,16		$C_{35}H_{62}O_3$	NR	530	26
epoxymurin-B, C15=C16 (epoxy-acetogenins, epoxymurin-A type)			$C_{35}H_{62}O_3$	NR	530	27
epomuricenin-B, C17=C18	13-14		$C_{35}H_{62}O_3$	NR	530	27
diepomuricanin-A (diepomuricanin- A type)	15,16,19,20		$C_{35}H_{62}O_4$	NR	546	27
Corepoxylone, (diepomuricanin- A type)	15,16,19,20		$C_{35}H_{60}O_5$	NR	560	27
Solamin, (uvariamicin-1 type)	15,20	threo-trans-cis	$C_{35}H_{64}O_5$	Cytotoxic	564	27
Murisolin, (murisolin type)	4,15,20	threo/trans/threo	$C_{35}H_{64}O_6$	Cytotoxic	580	27
Corossolin, (murisolin type)	10,15,20	threo/trans/threo	$C_{35}H_{64}O_6$	Cytotoxic	580	27
Corossolone, (C=O,10) (murisolin type)	15,20	threo/trans/threo	$C_{35}H_{62}O_6$	Cytotoxic	578	27
Annomutacin, (annonacin type)	4,10,17,22	threo/trans/erythro	$C_{37}H_{68}O_7$	Cytotoxic	624	23
cis-annonacin, (annonacin type)	4,10,15,20	threo/trans/threo	$C_{35}H_{64}O_7$	Cytotoxic Insecticidal Anti-microbial, anti-tumour, neurotoxic, neurodegenerative.	596	28

Acetogenins and Olefinic position	Hydroxyl positions	Relative configuration	Molecular formula	Bioactivity	M^+	References
cis-annonacinone, (C=O,10) (annonacin type)	4,15,20	threo/trans/threo	$C_{35}H_{62}O_7$	Cytotoxic	594	28
cis-goniothalamicin, (annonacin type)	4,1O, 13,18	threo/cis/threo	$C_{35}H_{64}O_7$	Cytotoxic	596	28
arianacin +javoricin+ (+;12-epimer for the authors), (annonacin type)	4,12,15,20	threo/cis/threo	$C_{35}H_{64}O_7$	Cytotoxic	596	28
annomuricin-A, (annominicin type)	4,10,11,15,20	threo-threo/trans/ erythro	$C_{35}H_{64}O_8$	Cytotoxic	612	27
annomuricin-B, (annomonicin type)	4,10,11,15,20	erythron-threo/trans/ erythro	$C_{35}H_{64}O_8$	Cytotoxic	612	27
muricatin-C, (C=O,10) (annomonicin type)	4,15,20,25	threo/trans/threo	$C_{35}H_{62}O_8$	NR	610	27
muricatocin-A (annomonicin type)	4,10,12,15,20	Pseudo erythro-threo/trans/threo	$C_{35}H_{64}O_8$	Cytotoxic	612	29
muricatocin-B (annomonicin type)	4,10,12,15,20	Pseudo erythro-threo/trans/erythro	$C_{35}H_{64}O_8$	Cytotoxic	612	29
muricatocin-C (annomonicin type)	4,10,12,15,20	Pseudo threo-threo/trans/erythro	$C_{35}H_{64}O_8$	Cytotoxic	612	30
annomuricin-C (annomonicin type)	4,10,11,15,20	threo-threo/trans/threo	$C_{35}H_{64}O_8$	Cytotoxic	612	30
Annohexocin (annomonicin type)	4.8,10,12,15,20	threo/trans/erythro	$C_{35}H_{64}O_9$	Cytotoxic	628	16
Muricatalicin (annomonicin type)	4,7,13,15,20	threo/trans/threo	$C_{35}H_{64}O_8$	NR	612	31

NR: not reported.

Figure 3. The extrinsic cytochrome c pathway promotes caspase-8, whereas the intrinsic pathway promotes caspase-9. Unless these initiator caspases are triggered, activation of caspases 7, 6, and 3 initiates the apoptosis execution process. Cell apoptosis is predicated on the expression of caspase protease, and caspase-3 activation is a critical downstream consequence.

Cytotoxic Activity

One of the key factors in the development of cancer is a malfunctioning apoptotic pathway. Apoptosis, a mechanism that rids the body of cancerous cells, has been related to several tumour types, including breast, pancreatic, ovarian, and colorectal tumours.[33-37] By causing cell viability loss, morphological alterations, membrane mitochondrial potential decline, and cell arrest in the G0 or G1 phase, *A. muricata* display antiproliferative effects on a variety of multidrug resistant tumour cell lines.

The most researched tumour cell lines using *A. muricata* preparations *in vitro* are: HaCat, or immortalised human keratinocytes, breast cancer cells; MDA-MB-435S; Bovine cell line MBDK; WRL-68, normal human liver cells; FG or COLO357 and CD18 or HPAF, pancreatic tumour cells; U937, histiocytic lymphoma cell line; MCF-7, human breast carcinoma; CV304, human leukaemia carcinoma cells; the cervical tumour cell line HeLa; human big lung cell cancer; human bladder carcinoma; human bladder carcinoma cells; HT-29 and HCT-116, colon tumour cells; VERO, kidney epithelial cells; C-678, stomach tumour cells; S-F-268, glioma; CCD841, normal human colon epithelial cells; EACC stands for Ehrlich ascites carcinoma cells, and SKBR3 for breast adenocarcinoma cell line. T47D stands for breast tumour cells, while HL-60 stands for human promyelocytic leukaemia. The rising usage of *A. muricata* as a tumour preventative therapy mentioned in ethnobotanical records may be connected to its specific cytotoxic properties.[38] Table 2 displays the *in vitro* cytotoxic activity of several constituents of *A. muricata* extracts.

Annona muricata against Human Breast Cancer Cell Line

Many researches have shown the possible utility of this herb in therapeutic treatments for breast cancer, specifically. Preparations

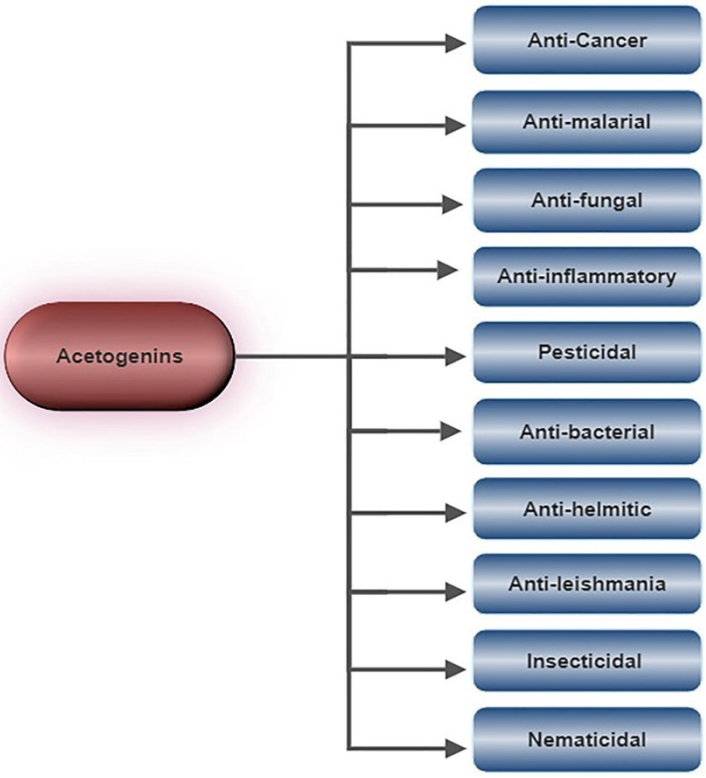

Figure 2: Major bioactivities of acetogenins.

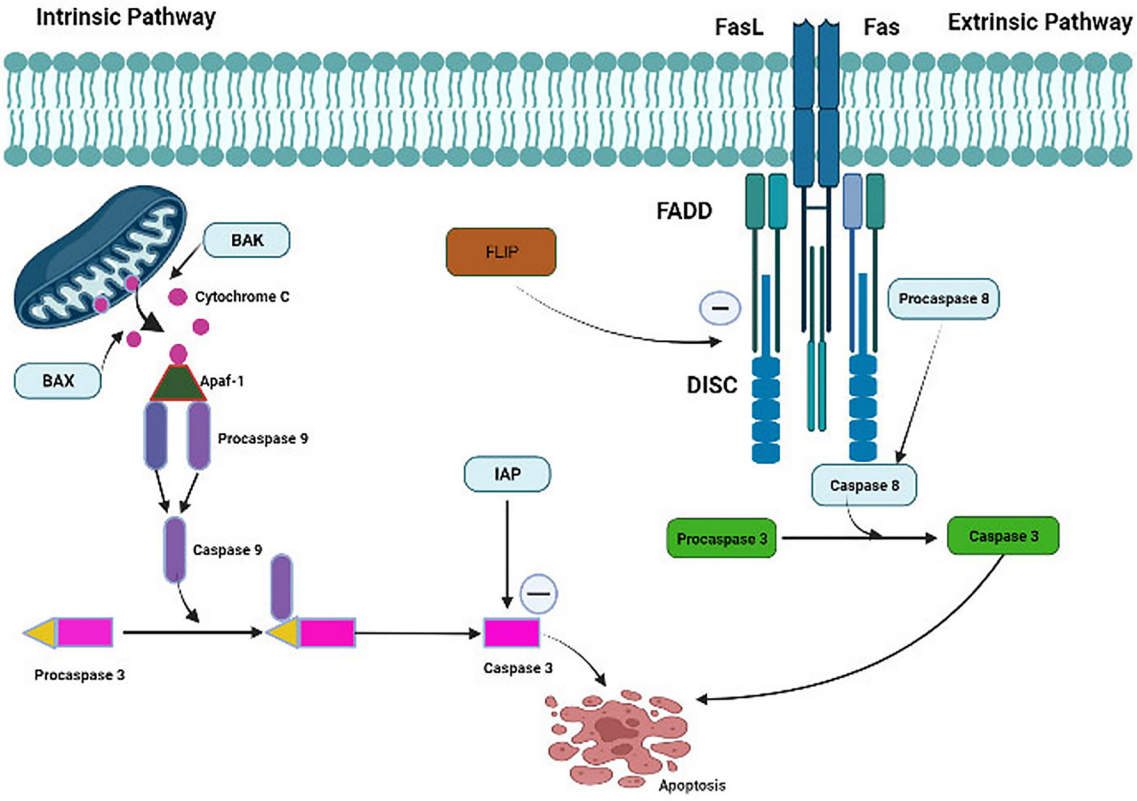

Figure 3: Mechanism of apoptosis. Apaf-1: Apoptosis protein activatory factor-1, Fas: Fas receptor, BAX and BAK: Apoptosis regulator proteins, DISC: Death inducing signalling complex. IAP: Inhibitors of apoptosis protein, FADD: Fas associated death domain.

from *A. muricata* have been shown to suppress the proliferation of breast tumour cells during patient treatment operations by instigate cytotoxic effect in lung tumour cell lines. This event, which results in an imbalance between cell growth and death, can be brought on by apoptotic pathway malfunctions. Identical to other malignancies, breast tumour cells may be unable to undergo apoptosis because of changes in the apoptotic pathway. For instance, breast cancer development has been associated with abnormalities in the intrinsic pathway. Deficits in the management of cytochrome release,[55] apoptosome formation,[56] and caspase stimulation have been seen in breast tumour cells.[57]

The capability of *A. muricata* leaf methanol extracts (LMEAM) to trigger apoptosis and/or halt cell cycle development in MCF-7 breast tumour cells was investigated by Naik *et al.*[58] This study revealed that LMEAM can suppress MCF-7 tumour cells in a dose-dependent mode without harming normal breast tumour cells. This suggests that LMEAM may preferentially target particular processes in particular tumour cells, as has been documented in the literature.[11,59-61] Hoescht 33342 staining revealed chromatin condensation, DNA fragmentation, and a decrease in cell number in response to LMEAM therapies, while control cells showed normal cellular design with no obvious changes. This evidence suggests that *A. muricata* preparations caused morphological alterations in MCF-7 cells that favour apoptosis.[62] Naik *et al.* investigated the distribution of MCF-7 cells across the cell cycle to see if LMEAM induced growth suppression regulates cell cycle arrest. S-phase and G2/M-phase cells were discovered to be in the proliferative stages, but sub-G1-phase was the definition of apoptosis. The results of this study showed that LMEAM causes the cell cycle to stop at the G1 phase, which was preceded by a modest improvement in sub-G0 or G1 phase cells and a massive reduction in S-phase cells, indicating that LMEAM treatment produced a blockage effect in the G1 or S transition due to apoptosis activation and sub-G0 or G1 cell cycle arrest.[62]

MCF-7 cells treated dose-dependently with LMEAM revealed an elevation in early and late apoptotic cells when stained with Annexin V-PI. Additionally, Naik *et al.* discovered bi-phasic effects that were unrelated to dose impacts, with a rise in the number of necrotic cells at a lower dosage (50 g/mL) and a reduction at a higher dosage (100 g/mL).[58] This bi-phasic response has been observed in several anticancer drugs and has been thoroughly documented using *A. muricata* ethyl acetate extracts and an isolated acetogenin, 15-acetyl guanacone.[63]

Annona muricata against Human Caucasian Promyelocytic Leukaemia Cell Line

The National Cancer Institute (NCI), USA which recommended using an inhibition zone lower or same to 20 gm/mL as a standard for appropriate screening tumour medications from medicinal plants and herbs, a study by Constant Anatole Pieme *et al.* reported that extracts of *A. muricata* substantially inhibited the HL-60 cells *in vitro*. This suggests that the extracts are active.[53] Extracts had a dose dependent inhibitory impact in HL-60 treated cells as well. The roots outperformed the other extracts in terms of cytotoxicity. Hu W *et al.* 2010 found that after treatment for 24 hr using *A. muricata* extracts, DNA fragmentations at 100 gm/

Table 2: *A. muricata* extracts and their *in vitro* cytotoxic activity.

Plant part	Solvent	Cell line	Effect	References
Leaf	Water: ethanol (40%)	K562, ECV- 304	MIC*=7 mg/mL MIC=2 mg/mL	39
Pericarp	Methanol Hexane Ethyl acetate	U-937	MEC*>1 mg/mL MEC=1 mg/mL MEC=0.1 mg/mL	19
Dried fruit	Water: acetone (50%)	MCF-10A BC MDA-MB-468 MDA-MB-231 MCF-7	MIC*>200 µg/mL MIC=4.8 µg/mL MIC>200 µg/mL MIC>200 µg/mL	40
Leaf Stem	Ethyl acetate Ethyl acetate Methanol Hexane Ethyl acetate Methanol Hexane	U-937	MLD*=7.8 µg/mL MIC=10.5 µg/mL MIC=60.9 µg/mL MIC=18.2 µg/mL MIC=28.1 µg/mL MIC=38.5 µg/mL MIC=15.7 µg/mL	41 42
Leaf	Ethanol	VERO H460 C-678	MIC<0.00022 mg/mL MIC<0.00022 mg/mL MIC<0.00022 mg/mL	43

Plant part	Solvent	Cell line	Effect	References
Leaf/stem leaf	DMSO	PC FG/COLO357	MIC=200 µg/mL	44
	Butanol	PC CD18/HPAF	MIC=73 µg/mL	38
	Water: ethanol	MDA-MB-435S	MIC=29.2 µg/mL	45
	Water	HaCaT	MIC=30.1 µg/mL	46
	Ethanol	WRL-68	MIC=52.4 µg	47
	Pentane	HaCaT	1.6 to 50 µg/mL increase cellular activity,	
	Ethanol	A375	100 µg/mL does not change cell behaviour	
		MCF-7	MIC>500 µg/mL	
		H-460	MIC=320 µg/mL	
		SF-268	MIC=140 µg/mL	
			MED*=6.2 g/mL	
			MED=4.0 µg/mL	
			MED=8.5 µg/mL	
Leaf	Ethanol	MDBK	MCC*=20x10-4 lg/mL	48
Seed	Ethyl acetate	HeLa	MCC=24x10-5 lg/mL	49
Leaf	Ethanol+ water	HT-29	15.62 µg/mL=11.37% inh	50
	Chloroform	HCT-116	15.62 µg/mL=3.97% inh	51
	n-Hexane	CCD841	15.62 µg/mL=18.42% inh	52
	n-Hexane	Spleen cell	15.62 µg/mL=21.41% inh	
	Ethyl acetate	EACC	MIC=14.93 µg/mL	
	Methanol	MDA	MIC=4.29 µg/mL	
	n-Hexane	SKBR3	MIC>100 µg/mL	
	Ethyl acetate	T47D	MIC=12.26 µg/mL	
	Methanol		MIC=3.91 µg/mL	
	n-Hexane		MIC>100 µg/mL	
	Ethyl acetate		MIC=42.19 µg/mL	
	Methanol		MIC=34.24 µg/mL	
	Ethanol		MIC>100 µg/mL	
			MIC>750 µg/mL	
			MIC=335.85 µg/mL	
			MIC=248.77 µg/mL	
			MIC=202.33 µg/mL	
			MIC=17.15 µg/mL	
Leaf	Ethanol	HL- 60	MIC=14 µg/mL	53
Twigs	Hexane	Capan-1	MIC=49 µg/mL	54
Roots	DMSO		MIC=9 µg/mL	
Leaf			MIC=7.8 µg/mL	
Com leaf			MIC=0.9 µg/mL	

***MIC:** minimum inhibitory concentration, **MEC:** minimum effective concentration, **MLD:** median lethal dose, **MED:** median effective dose, **MCC:** median cytotoxic concentration.

mL were detected and the intensity of Hoechst 33258 staining, which is a crucial indicator of apoptosis, increased.[64]

Based on the findings of Constant Anatole Pieme *et al.*, believe that extract of *A. muricata* induce HL-60 cell apoptosis. By rupturing mitochondrial membranes, *A. muricata* preparations stop cells in the G0 or G1 phase and slow down cell development.[65,66] Checkpoints in the cell cycle are regulatory mechanisms that ensure proper cell cycle progression. Myung-Ja Y *et al.* demonstrated in an *in vitro* apoptosis experiment that preparations from *A. muricata* caused HL-60 tumour cells to undergo apoptosis in a dose-dependent way as opposed to untreated cells.[67] After a 24 hr treatment period, *A. muricata* preparations triggered G0 or G1 cell cycle inhibition in HL-60 cells at various dosages. One may hypothesise that the G0 or G1 cell cycle halt and cell

differentiation are connected to the anticancer properties of *A. muricata* preparations.[67] These cell cycle studies showed that all this plant extracts could considerably stop the G0 or G1 phase in HL-60 cells, but they had little effect on the G2/M phase.

Annona muricata Against Human Mammary Carcinoma, 4 T1 and MCF- 10A Cell Lines

The MCF-10A, 4 T1, and human mammary carcinoma, cell lines were more specifically impacted by the aqueous leaf extract of soursop samples, according to the cytotoxicity profile discovered by Syed Najmuddin *et al.*, which is consistent with the notion of treating breast tumour cells.[68] The *A. muricata* leaf aqueous extract sample with the greatest IC_{50} profile was used to continue treating the 4 T1 cells. According to study,[68] treatment with an aqueous extract was less toxic to normal cells since it needed a higher dose to kill them (Inhibitory activity=1000 g/mL), which was four times higher than the Inhibition zone of the group treated with an aqueous extract in 4 T1 cells. This finding points to the low toxicity of *A. muricata* crude extract. A flow cytometric examination of Annexin V or FITC at 48 and 72 hr separated a population of early apoptotic, late apoptotic/necrotic, and surviving cells because of Annexin V's high affinity adhering to Phosphatidylserine (PS), a phospholipid element of the cell membrane.[68]

A physiological change that occurs in dying cells during apoptosis causes phosphatidylserine to externalise to the outside of the plasma membrane leaflet. The aqueous leaf extract specimen treatment group showed a greater overall apoptosis percentage than the untreated group when comparing premature apoptotic and delayed apoptotic/necrotic cells. It supports studies that indicate soursop can induce apoptosis in colon cancer cells.[50] The aqueous sample treatment produces apoptosis in a time-dependent way, with apoptotic cells shown to be greater in the 72 hr timepoint than in the 48 hr timepoint. Since Annexin V/FITC analysis depends on PS externalisation, this AO or PI (Acridine Orange or Propidium Iodide) test was developed specifically to identify distinct cellular processes or morphological characteristics in cells treated by aqueous extract of *A. muricata* sample. The ability of the soursop aqueous extract to induce apoptosis and suppress breast cancer cells was shown by the treated 4 T1 cells' AO or PI staining, which showed apoptotic characteristics as membrane blebbing, nucleus shrinkage, and DNA fragmentation.[69] The amount of 4 T1 breast cancer cells that spread to secondary locations, such the lung organ of the tumour-bearing mice, was reduced in the extract-treated group, as shown by the drop in colonies produced in the clonogenic experiment. The application of the aqueous extract altered the morphology of the colony development.[70] This implies that the presence of aqueous extract treatment causes 4 T1 tumour cells to become less migratory and more adherent to one another. Cell-cell adhesion and cell migration may be related to the formation of colonies from cell ensembles.

Annona muricata against Pancreatic MIA PaVa-2, HT-29 and HepG2 cell lines

It has been discovered that several acetogenins (annomuricins A, B, C, and E) are toxic to different kinds of cancer cells. Cancer cells from the pancreatic MIA PaCa-2, colon HT-29, or lung A549 are all harmful to the plant's leaves.[7,19,23,30] In a study by Wu *et al.*, the extract was prepared at room temperature using 75% ethanol as the solvent, and after 24 hr of treatment, the IC_{50} for inhibiting proliferation in HepG2 liver cancer cells was 150 g/mL.[29,71] This demonstrates that 75% ethanol is an excellent solvent for extracting anticancer compounds from herbs. But when compared to other extraction solvents, ethyl acetate appears to be the best. Lung A549, colon HT-29, or pancreatic MIA PaCa-2 cancer cells are toxic to the annomuricins A, B, C, and E found in *A. muricata* leaves.[7,19,23,30] The plant's leaves also contain murihexocin C and muricoreacin, which are poisonous to cells that cause Pancreatic Cancer (PACA-2) and Prostate Adenocarcinoma (PC-3) respectively.[7]

DISCUSSION

In the review by Moghadamtousi and colleagues, acetogenins are included, some of which have been demonstrated to be toxic to cancer cells.[12] There is evidence that acetogenins, in contrast to being toxic to tumour cells, can have unacceptably severe side effects such neurotoxicity, which can result in neurodegeneration.[22] Acetogenins are thus unlikely to be effective therapeutic agents unless chemical alteration can preserve the apoptotic activity while reducing that neurotoxicity.[72] Because active ingredients in extracts may have additive effects, their anticancer impact and mechanism may differ from those of a single chemically defined substance.[73]

As the dysregulation of the cell cycle is directly associated to apoptosis, cell cycle analysis was also carried out after that.[40] In order to maintain the integrity of the cell, the regulation of the cell cycle uses a number of checkpoint pathways to make sure that one phase of the cell cycle is finished before moving on to the next.[74] The fact that the percentage of cells in the sub G0 or G1 phase increased significantly suggests that the aqueous extract-treated group experienced cell cycle arrest. It is compatible with the idea of treating tumour cells to stop the tumour cells' cell cycle, which finally ends in cell death.

CONCLUSION

In traditional medicine, *A. muricata* is frequently used to treat ailments including cancer, hypertension, inflammation, diabetes, diarrhoea, dysentery, and fever as well as pain, respiratory and skin conditions, parasitic infections both internal and external, and bacterial infections. The most popular preparations are decoctions made from bark, roots, seeds, or leaves. Although clinical evidence is sparse, *in vitro* and *in vivo* studies provide support for most traditional uses. Treatments for obesity, their

effectiveness in treating conditions of the respiratory system, heart, and kidneys, as well as therapies for animal bites and stings, are some traditional applications that have not yet received scientific confirmation.

Researchers are still interested in the biological features of annonaceous acetogenins due to its selectivity. Out of more than 200 phytochemicals from various parts of *A. muricata*, more than 120 acetogenins have been isolated. Although the precise mechanism by which *A. muricata* extract causes cell differentiation is uncertain, differentiation may be associated with a halt in cell cycle progression in the G0 or G1 phase. For this reason, earlier research has found that certain acetogenins operate as a DNA topoisomerase I toxin, prevent cancer cells from entering their G1 phase, activate pathways linked to Bax-Bak and caspase-3, and block NADH-ubiquinone oxidoreductase (Complex I) in mitochondria.

After carefully examining all of the information, we can conclude that natural treatments for the majority of illnesses, including cancer, are thriving in our own backyard, particularly in the tropics. The separation of a single active molecule from extracts may result in increased toxicity as well as a reduction in therapeutic effectiveness. In addition to the *in vitro* study, further *in vivo* tests are required to demonstrate these pathways.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

THF: Tetrahydrofuran; **DISC:** Death inducing signaling complex; **IAP:** Inhibitors of apoptosis protein; **FADD:** Fas associated death domain; **Apaf-1:** Apoptosis protein activatory factor-1; **LMEAM:** *A. muricata* leaf methanol extract.

SUMMARY

A. muricata is a popular traditional medicine used to treat various ailments, including cancer, hypertension, inflammation, diabetes, diarrhea, dysentery, fever, and more. Decoctions made from bark, roots, seeds, or leaves are popular preparations. While clinical evidence is limited, *in vitro* and *in vivo* studies support most traditional uses. Some traditional applications, such as obesity treatments, respiratory system conditions, heart and kidney conditions, and animal bites and stings, have not received scientific confirmation. Researchers are interested in the biological features of annonaceous acetogenins, which have been isolated from over 200 phytochemicals. Some acetogenins function as DNA topoisomerase I toxins, prevent cancer cells from entering their G1 phase, activate pathways linked to Bax-Bak and caspase-3, and block NADH-ubiquinone oxidoreductase in mitochondria. Natural treatments for most illnesses, including cancer, are thriving in tropical regions.

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